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## THE CHROMOSOMES IN CROSS-FERTILIZED ECHINOID EGGS.

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In experiments which I carried on during the summer of 1907<sup>1</sup> crosses were made between several echinoids, namely, by the fertilization of :

1. The egg of the spatangoid *Moira atropos* with the sperm of the sand-dollar *Mellita pentapora*.
2. The egg of *Moira* with the sperm of the sea urchin *Toxopneustes variegatus*.
3. The egg of *Moira* with the sperm of the sea urchin *Arbacia punctulata*.
4. The egg of *Toxopneustes* with the sperm of *Moira*.
5. The egg of *Toxopneustes* with the sperm of *Mellita*.
6. The egg of *Arbacia* with the sperm of *Moira*.
7. The egg of *Arbacia* with the sperm of *Mellita*.
8. The egg of *Mellita* with the sperm of *Moira*.

The work was undertaken primarily with the object of obtaining material for a cytological study of cross-fertilized eggs and secondarily for the purpose of making a comparison, based especially upon the character of the skeleton, between larval forms.

In this paper I shall consider some of the earlier phenomena exhibited, in brief, the prophases and early metaphases of division, in two of the crosses, (1),  $\frac{\text{Moira } \delta}{\text{Toxopneustes } \varphi}$  and (2),  $\frac{\text{Moira } \delta}{\text{Arbacia } \varphi}$ , reserving the consideration of later stages, of the other crosses, and a general discussion of the results for a latter contribution.

The method of effecting the cross-fertilization was the exceedingly simple one of allowing the eggs, after their removal from the ovary, to stand for several hours in sea water, the water being changed occasionally, and at the most favorable time, which was

<sup>1</sup> I wish to express my thanks to the Hon. George M. Bowers, U. S. Commissioner of Fisheries, for the privilege of working in the Beaufort Laboratory and to Mr Henry D. Aller, director of the laboratory, for many courtesies extended to me. I am also indebted to Dr. Bartgis McGlone for information regarding the artificial fertilization of *Moira* eggs.

determined by experiment, to fertilize the eggs with normally very active sperm.

All attempts at cross-fertilization of the eggs immediately after their removal from the ovary were unsuccessful.

For the  $\frac{\text{Moira } \delta}{\text{Toxopneustes } \varphi}$  crosses the eggs were allowed to stand in sea water for five hours and for the  $\frac{\text{Moira } \delta}{\text{Arbacia } \varphi}$  crosses for seven hours, before fertilization.

Fully 95 per cent. of the eggs so treated underwent a regular and comparatively uniform cleavage, the greater number developing into swimming blastulæ and gastrulæ. About 75 per cent. of these embryos never developed beyond this stage. The remaining 25 per cent. developed into plutei which remained alive and were kept under observation for about ten days.

This high percentage of segmentation was never approached in experiments in which chemicals were employed as aids in effecting cross-fertilization, although naturally no attempts to bring such methods to perfection were made after I had obtained so simple a means of bringing about the results that I desired.

The fact that the eggs were actually fertilized was recognized in the transparent *Toxopneustes* eggs by the observation of the union of the pronuclei. In the cases of both the *Toxopneustes* and *Arbacia* eggs a fertilization membrane was formed.

Each series was checked by a control series of unfertilized eggs. In these controls the eggs were allowed to stand, with occasional changes of sea water, and in every instance the eggs ultimately disintegrated without undergoing segmentation.

The figures that illustrate the account that follows were drawn from sections of picro-acetic and sublimate-acetic material stained in iron hæmatoxylin.

#### A. THE *Moira* $\delta$ *Toxopneustes* $\varphi$ CROSS.

The *Toxopneustes* eggs stood in sea water, which was changed four times, for five hours when they were fertilized with *Moira* sperm.

Cleavage began 40-45 minutes later. The time consumed during the entrance of the spermatozoon, fusion of the pronu-

clei, formation of the amphiaster, etc., is then approximately the same as in *Toxopneustes* eggs fertilized with *Toxopneustes* sperm.

The chromosomes as seen in a polar view of the equatorial plate of  $\frac{\text{Toxopneustes } \delta}{\text{Toxopneustes } \varphi}$  eggs are shown in Figs. 1 and 2.

They are seen to have the appearance of rather long, slender, and somewhat bent rods. By comparing these two figures it may be seen that variations in the form of the chromosomes, which are correlated with slight differences in the ages of the plates, are evident.

A corresponding view of the chromosomes in a section of a  $\frac{\text{Moira } \delta}{\text{Moira } \varphi}$  egg is shown in Fig. 3.

Some differences are apparent, but in general the size, form, etc., of the chromosomes in this plate are so like those of the *Toxopneustes* egg that one need scarcely venture to hope to be able to identify the chromosomes of maternal and paternal origin in the cross-fertilized eggs.

An examination of sections such as are illustrated in Figs. 4 and 5 convinces me that we have here a mixture of the two sorts, but I find myself unwilling or perhaps unable to distinguish the chromosomes of either origin.

Some interesting variations from the normal were found in one series of  $\frac{\text{Moira } \delta}{\text{Toxopneustes } \varphi}$  eggs in which more than one spermatozoon had entered the egg. Two different results are shown in Figs. 9-12 and Text Fig. 1.

In one case the extra sperm-nucleus is seen moving toward the segmentation nucleus. Its aster has divided while the centrosome of the future cleavage amphiaster is still single (Fig. 9). Later the second sperm nucleus seems about to fuse with the segmentation nucleus while the centers of the regular cleavage amphiaster have separated (Fig. 10). In some cases fusion between the two nuclei takes place; in others (Fig. 11), the fibers from one of the sperm asters enter the nucleus and the chromosomes become differentiated in the network before the cleavage asters have well separated.

In the other case (Fig. 12 and Text Fig. 1), chromosomal

differentiation and separation of the cleavage centers had gone on to a considerable extent before the additional spermatozoon had entered the egg. Here the two amphiasters are seen side by side. In Text Fig. 1 what may possibly be sperm tails are seen lying within a fertilization cone, although the entrance of the tail

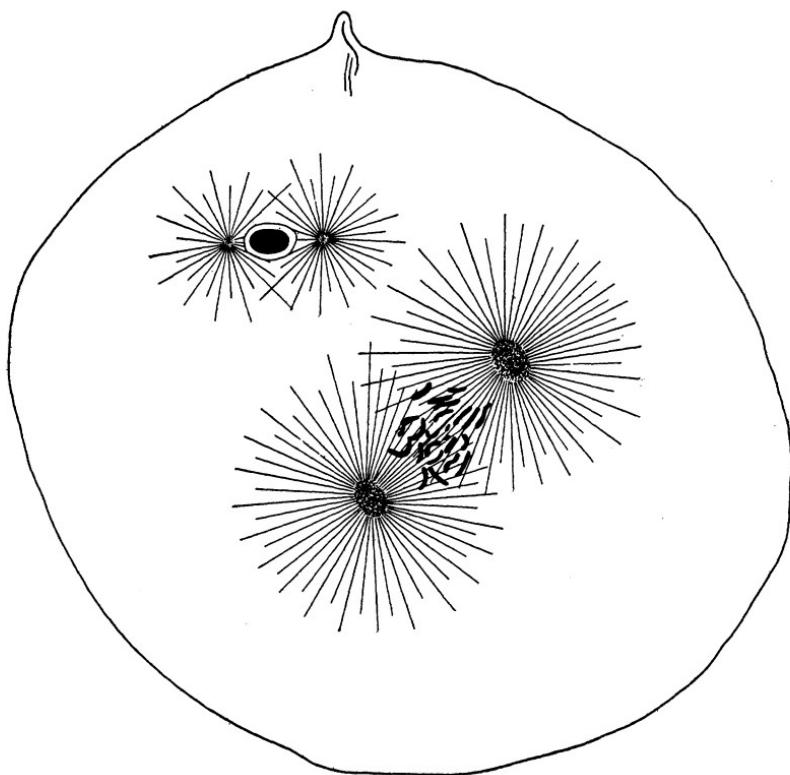


FIG. 1. *Toxopneustes* egg  $\times$  *Moira* sperm. (Drawn to same scale and reduced slightly more than are plate figures.) Segmentation nucleus dividing. Extra sperm nucleus in prophase.

in the fertilization of the echinoderm egg is contrary to the general belief.

These cases ought to prove of interest in further investigation along the lines laid down by Boveri in his recent contribution on dispermic sea urchin eggs (*Zellen-Studien*, Heft 6).

B. THE *Moira* ♂ *Arbacia* ♀ CROSS.

In effecting this cross the *Arbacia* eggs were allowed to stand in sea water for seven hours, the water being changed every hour, and then fertilized. The controls gave no segmentation.

Cleavage began about forty minutes later; again approximately as in normally fertilized eggs, in both cases being slightly hastened or retarded by variations in the temperature of the water.



FIG. 2. *Arbacia* egg  $\times$  *Moira* sperm. Egg outline omitted, otherwise drawn to same scale and reduced as are plate figures. Chromosomes scattered throughout cytoplasm. *Arbacia* chromosomes and *Moira* chromosomes may be distinguished from one another by size.

The sections of eggs of this cross are perhaps of greater interest than those of the *Moira-Toxopneustes* cross because of the fact that the chromosomes of the two species are of sufficient difference in form to be distinguished from one another.

Fig. 6 shows the chromosomes of an equatorial plate of an *Arbacia* ♂ egg. The chromosomes here are seen to be short, slightly bent rods. These are quite different in form from those already mentioned in the equatorial plate of *Moira* (Fig. 3), where the chromosomes are longer and comparatively more slender.

The sections of the *Moira-Arbacia* cross-fertilized eggs giving a polar view of the equatorial plates (Figs. 7 and 8), show a mixture of short and long forms probably indicating *Arbacia* and *Moira* chromosomes respectively. These differences in form are evident in the equatorial plates of both the first and second cleavages, which is as far as I have carried the observations.

The differences in form are less evident in the late metaphases or early anaphases when the daughter chromosomes are drawn out, behaving like substances with a high surface tension, and then contracting during the late anaphases, into much shorter rods.

In both of the crosses, but especially in sections of eggs of the *Moira-Arbacia* cross, an interesting phenomenon may be noted (Text Fig. 2).

In eggs in which the daughter nuclei are in the resting condition succeeding the first division, the cytoplasm contains many deeply staining rods. The nucleus at this time does not take the chromatin stain and appears like an empty vesicular structure.

In eggs, of the same lot and on the same slides, in which the fibers of the second amphiaster have begun to form, the nucleus again takes the stain and shows the chromatic net, while the cytoplasm is seen to be free from the bodies described.

These structures have puzzled me not a little, but I have finally reached the conclusion that the eggs in which they occur are degenerating. Even though this be true it is difficult to explain the simulation or perhaps occurrence of longitudinal and transverse divisions of these chromosomes lying free in the cytoplasm.

#### SUMMARY.

This paper deals with observations made on sections of cross-fertilized eggs of two kinds: (1) *Toxopneustes* eggs fertilized with *Moira* sperm, (2) *Arbacia* eggs fertilized with *Moira* sperm.

The results of the study may be summarized as follows:

1. The equatorial plate of the *Moira-Toxopneustes* cross shows a mixture of two kinds of chromosomes not sufficiently unlike one another to enable a positive distinction between the two.
2. The equatorial plate of the *Moira-Arbacia* cross shows a mixture of two kinds of chromosomes, one variety long, the other variety short. These differences in form are correlated with the spermatozoon and the egg respectively.

BRYN MAWR COLLEGE,  
March, 1908.

## EXPLANATION OF PLATE I.

All of the figures were drawn with the aid of a camera and Zeiss compensation ocular 12 and 2 mm. Apochromatic oil immersion objective. They were enlarged two diameters with a drawing camera and have been reduced to one half in reproduction.

FIG. 1. *Toxopneustes* egg  $\times$  *Toxopneustes* sperm. Equatorial plate. Polar view.

FIG. 2. Same as Fig. 1.

FIG. 3. *Moira* egg  $\times$  *Moira* sperm. Eq. pl.

FIG. 4. *Toxopneustes* egg  $\times$  *Moira* sperm. Eq. pl.

FIG. 5. Same as Fig. 4.

FIG. 6. *Arbacia* egg  $\times$  *Arbacia* sperm. Eq. pl.

FIG. 7. *Arbacia* egg  $\times$  *Moira* sperm. Eq. pl.

FIG. 8. Same as Fig. 7.

FIG. 9. *Toxopneustes* egg  $\times$  *Moira* sperm. Segmentation nucleus with centrosome undivided. Extra sperm nucleus with aster divided.

FIG. 10. *Toxopneustes* egg  $\times$  *Moira* sperm. Segmentation nucleus with centrosome divided. Extra male nucleus, with its aster divided, in contact with segmentation nucleus.

FIG. 11. *Toxopneustes* egg  $\times$  *Moira* sperm. Segmentation nucleus with centrosome divided. Fibers from aster of extra sperm nucleus extending into the segmentation nucleus.

FIG. 12. *Toxopneustes* egg  $\times$  *Moira* sperm. Segmentation nucleus and extra sperm nucleus lying side by side and both preparing for division.

